Recurrent Half-Sib Selection with Testcross Evaluation for Increased Oil Content in Soybean

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ABSTRACT

Protein meal and oil are the two commodities produced from soybean [*Glycine max* (L.) Merr.] that give the crop its value. Increasing seed concentrations of either or both may add value. Objectives of this study were to investigate the effectiveness of recurrent half-sib selection for increased seed oil, to evaluate the effect of tester oil content on selection response, and to investigate testcross heterosis and inbreeding depression for seed oil content. A recurrent half–sib selection system was devised for soybean and selection for increased oil content was conducted in a population for seven and three cycles using a high and a low-oil tester, respectively. The base population was a high-oil composite with gray pubescence (*tt*) that was segregating for nuclear genetic ms_1 male sterility. In summer, the base population was planted in single plant hills and bordered with the tester (Ms_1Ms_1TT) in a random mating block in North Carolina. About 100 to 200 random male-sterile plants with hybrid seeds were harvested. Half-sib families derived from each male-sterile plant were then grown in Puerto Rico in winter. At maturity, seeds from tawny plants (tester hybrid) were used to identify half-sib families with high-oil content. Corresponding gray plant hybrids from sib matings within the population were bulked to start the next cycle of selection. Random progenies from the base populations and selected progenies from each cycle of selection were evaluated in a replicated field experiment at three locations in North Carolina. Cycle × tester hybrids and cycle × cycle sib hybrids were also included in the tests. The results showed that oil content was significantly increased at a rate of 1.1 ± 0.2 g kg^{-1} cycle^{-1} in the high-oil tester populations but not in the low-oil tester populations. The realized heritability estimate for the high-oil tester population was 0.12 ± 0.03. Evidence of heterosis indicated that some dominance effects on oil content existed. Dominance effects may affect the evaluation accuracy of the genotypes being tested. A high-oil tester and high-oil populations may have many common alleles resulting in less dominance and more additive effects in their hybrids. Consequently, a high-oil tester can lead to better evaluation and selection precision, compared with a low-oil tester which could mask additive effects and reduce selection precision.

Protein meal and oil are the two commodities that give soybean its value. Increasing the seed concentration of both as a way to add value to the soybean crop has been the subject of numerous studies. Of the two, oil concentration has received less attention. It is often considered a byproduct of protein meal production. Yet about half of the soybean value is derived from oil and an increasing world demand for edible oils and a decreasing supply (Golbitz, 1999), suggest that oil productivity of soybean should be improved. Previous research indicated that seed oil concentration is controlled by multiple genes with additive inheritance (Brim, 1973). Broad sense heritability estimates (plot mean basis) for oil content are relatively high (Burton, 1987) and range from 0.47 to 0.89. (Johnson et al., 1955; Kwon and Torrie, 1964; Shorter et al., 1976; Smith and Weber, 1968). Because of this high heritability, Burton and Brim (1981) developed a recurrent selection procedure for the rapid increase in oil content using a population segregating for a male-sterile gene, ms_1. Using this method, they showed that three cycles of recurrent selection increased seed oil content 11 g kg^{-1} seed and found realized heritability estimates for within-family and mass selection to be 0.2 and 0.28, respectively.

In two populations derived from a base population segregating for ms_1 male sterility, Priadi (1993) compared half-sib family and single selfed-plant recurrent selection for oil content. Six cycles of recurrent selection resulted in a per cycle linear increase of 1.1 ± 0.3 g kg^{-1} for single-plant and 0.5 ± 0.2 g kg^{-1} for half-sib family selection. No correlation was seen between oil and other agronomic traits, although there was a nonsignificant linear decrease in protein content.

Recurrent selection, as practiced above, is rapid in terms of cycles per year. That the mean oil concentration increased indicates that the frequency of genes affecting that trait increased in the population. The problem with selection for seed composition alone is that agronomic quality of the population may decline as the seed component increases (Brim and Burton, 1979). A possible way to prevent this would be to use half-sib recurrent selection and employ a good agronomic cultivar as the tester. At the very least, the improved trait would be expressed in a more agronomic genetic background, i.e., the population tester cross. Possible advantage could be made of this with additional breeding.

Testcross evaluation of unadapted germplasm in soybean was proposed by Kenworthy (1980) as a way to determine the combining ability of plant introductions. Several studies of testcross evaluation of germplasm have been conducted using the F_1 generation (Lewers et al., 1998) or advanced generations (Reese et al., 1988; St. Martin and Aslam, 1986). Results on the importance of choice of tester, however, were mixed. Lewers et al. (1998) conducted F_1 generation testcross evaluation of soybean germplasm using male sterility (ms_6ms_6) and seedling marker W_1. Significant F_1 and F_2 midparent heterosis and inbreeding depression for oil content were observed. Hence, there may have been some dominance

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Abbreviations: HOT, high-oil tester; LOT, low-oil tester.

effects for oil content. The choice of tester was important in detecting the value of parental germplasm.

St. Martin and Aslam (1986) used two adapted lines and two plant introductions (PIs) with good agronomic characteristics as male testers crossed with six adapted lines and six PIs. Lack of female × male interactions for yield and oil content in the study indicated that the choice of tester was not critical. Since there were no differences in yield among their testcross progenies, they concluded that testcross evaluation was not even necessary. Similarly, Reese et al. (1988) used four testers crossed with nine visually desirable lines and 14 PIs selected for yielding ability. The F_2 bulk populations were evaluated at three locations. They concluded that the choice of tester was not important since no tester group appeared to have an advantage over another.

A recurrent half-sib selection procedure was devised using a population that was segregating for *ms1* male sterility and two testers, one with high-oil concentration and one with low-oil concentration. Seven and four cycles of half-sib selection were completed with these two testers, respectively. The objectives of this study were (i) to investigate the effectiveness of recurrent half-sib selection for increased oil content and evaluate changes in agronomic traits that might be correlated with selection, (ii) to evaluate the effect of tester oil content on selection response, and (iii) to investigate testcross heterosis and inbreeding depression for seed oil.

MATERIALS AND METHODS

Population Development

The lines selected as the base population for this study were derived from two previous consecutive recurrent selection programs for increased seed oil content. Those two programs of recurrent selection and the current one involved intermating facilitated by male sterility and natural insect-assisted pollination.

The germplasm for the first recurrent selection program, which was also the genetic basis for the subsequent recurrent selection program for increased oil content, was derived from a random mating among six lines, 'Arksoy', 'Ogden', 'Lee', 'Roanoke', D60-8107, and 'Jackson'. Roanoke, N45-745, Ogden and Biloxi were grandparents of D60-8107. 'CNS' and Biloxi were parents of N45-745. Random mating was facilitated by male sterility induced by *Tobacco ringspot virus* (Brim, 1973). Four cycles of S_1 progeny recurrent selection were undertaken and 10 lines with high seed oil content were selected and used as the base population for the second program.

For the second program, the 10 selected lines were manually mated with genetic male-sterile (*ms1ms1*) plants (Burton and Brim, 1981). Equal portions of F_1 seeds (*Ms1ms1*) from each of the crosses were composited to form the C_0 population. Each cycle of selection consisted of a step of mass selection followed by a step of within half-sib family selection for high seed oil. At the end of the third cycle, 22 plants with high seed oil content and gray pubescence color (*tt*) were selected from a total of 106 male-sterile plants. Seeds from the 22 selected plants were composited to form the base population, designated as C_a, for the current recurrent selection program. The genotypes in the C_a population were a mixture of *Ms1Ms1tt*, *Ms1ms1tt,* and *ms1ms1tt*.

Recurrent Half-Sib Selection Using Testers

Two testers were chosen for comparing the effects of tester oil content on selection response. One was a high-oil low-protein cultivar, Ransom, and the other was a low-oil high-protein line, N80-2177-2. N80-2177-2 was a line derived from the population, NC-1 (Burton and Brim, 1983). Both testers have tawny pubescence, which is controlled by a dominant gene (*TT*). Corresponding gray pubescence is controlled by recessive genes (*tt*). This trait was used as a marker to facilitate tester hybrid evaluation and sib hybrid recognition.

In summer 1981, the base population was planted in single plant hills and bordered with the Ransom tester (*Ms1Ms1TT*) in a random mating block at Clayton, NC. To minimize contamination from other pollen sources, random mating blocks were separated from adjoining soybeans at least 2 m of open space and no plants were harvested in the outside 2 m on all sides of the block. At maturity, the block was divided into 20 sub-blocks. About 10 male-sterile plants with seeds were randomly harvested from each subblock. Genotypes of the hybrid seeds were a mixed population of *Ms1ms1Tt, Ms1ms1tt,* and *ms1ms1tt*. This population was then grown in a winter nursery in Puerto Rico. Seeds harvested from male-sterile plants (half-sib families) in North Carolina were grown in progeny rows. At maturity, oil content of 10 g of seed from the tawny fertile Ransom hybrid plants (*Ms1ms1Tt*) was measured by nuclear magnetic resonance spectroscopy (NMR) at North Carolina State Univ. to identify high-oil content families (Brim et al., 1967). Seeds of the corresponding fertile gray sib-hybrid plants (*Ms1ms1tt*) within the rows were bulked to start the next cycle of selection. This bulked population, designated as C_0, was used as the parent population for C_1. Seven cycles of this recurrent selection for high-oil content were completed. Each cycle resulted in a gray population. These populations were designated as HOT populations because they were developed using a High-Oil Tester.

Another recurrent selection procedure for increased oil content was initiated, using C_4 of the HOT population as the parent population, C_0, and N80-2177-2 as a tester. Except for the change of tester, the same testing, selecting, and intermating procedures were used. This recurrent selection procedure was conducted for three cycles and the resultant populations were designated as LOT populations because they were developed using the Low-Oil Tester, N80-2177-2.

The average selection differential of the two procedures was 8.2 and 10.5 g kg^{-1}, respectively. The average number of progenies tested in each cycle was 162. In most cycles, between 10 and 14% were selected from each population (Table 1).

Field Evaluation of Selection Progress

The experimental materials used for evaluating selection progress were random progenies from the base population (C_a), the unselected C_0 population, and the selected progenies from each cycle of recurrent selection designated as C_1 to C_7. All of the HOT and LOT progenies were bulk selfed for two generations and only the fertile plants were harvested in the first generation of bulk selfing. In the second generation of selfing, C_4, C_5, C_6, and C_7 of the HOT populations were bordered with Ransom and C_2 and C_3 of the LOT populations were bordered with N80-2177-2 to produce tester hybrids (cycle × tester) and sib hybrids (cycle × cycle). The sterile plants with hybrid seeds were harvested separately for each population and those seeds consisted of cycle sib hybrids (*ms1ms1tt and Ms1ms1tt*) and cycle tester hybrids (*Ms1ms1Tt*).

Therefore, the materials used for evaluation were the second generation bulk-selfed selected progenies of C_a, C_0, C_1

through C_7 gray (*tt*) fertile plants, and C_4 through C_7 tester-hybrid plants for the HOT populations; and the second generation bulk-selfed selected progenies of C_0, C_1 through C_3 gray (*tt*) fertile plants, and C_2, C_3 tester-hybrid plants for the LOT populations. Equal quantities of seeds from the random selected progenies of each population were composited for each cycle to evaluate selection response. These composite populations were entries in the field experiment. A total of 21 entries were used in the evaluation experiment including the two testers, Ransom and N80-2177-2.

The experiment was conducted in 1997 at three North Carolina locations [Clayton, (plinthic kandidult), Plymouth, (typic umbraquult), and Windblow (arenic paleudult)], in a randomized complete block design with three replications at each location. Each entry was grown in a three-row plot, 5.8 m long by 0.97 m wide. Seeding rate was 26 seeds per meter in rows. At maturity, the center row of each plot was end-trimmed to 4.9 m and harvested for seed yield. Plants in the hybrid plots were separated into gray (sib hybrids) and tawny (tester hybrids) and threshed separately. Data were recorded on performance of the middle row of each three-row plot. Recorded agronomic traits included flowering (all plants in flower) and maturity date (95% of plant mature), plant height (5 plants/plot) and lodging (all plants), seed weight and plot yield. Seed weight, protein and oil content were measured separately for the sib-hybrid and tester-hybrid plants. Protein and oil content were analyzed by near infrared reflectance spectroscopy (NIR) at the USDA North Regional Research Center, Peoria, IL.

Statistical Analysis

The SAS GLM procedure (SAS Institute, 1990) was used for the statistical analysis. Locations were considered to be random effects, and genotypes (cycles) were considered fixed. To test for genotype effects, the Type III mean square of location × genotype was used as the error term. To test location effects, the Type III Rep within location mean square was used as the error term. Residual mean square was used for testing the effect of location × genotype interaction.

For testing progress in selection for oil content in the HOT population, variation among cycles was partitioned into linear and quadratic effects and these effects were tested with the genotype × location interaction mean squares. Since the cycle quadratic × location was significant for oil and protein content, a separate analysis of variance for each location was conducted and the residual mean square was used as an error term for testing differences among genotype effects. To evaluate change of rank in oil content among locations, Spearman's rank correlation analyses were performed (Steel et al., 1997). Since these analyses indicated that there were no significant changes ($p > 0.20$) in the rank of oil content among locations, a combined analysis for oil content was also performed over locations.

The SAS PROC REG procedure was used for regression of oil content on cycles of selection over locations and by locations for the HOT populations. Realized heritability estimates were calculated by regression of cycle means on accumulated selection differential. The oil increase was fit to a linear and quadratic model. The quadratic term was dropped from the regression model because it was not significant.

The SAS PROC CORR procedure was used for calculating simple correlation coefficients between oil content and other measured traits. The number of sterile plants per plot was used as a covariate in the initial analysis. Because the covariate was not significant, it was dropped from the analysis.

Table 1. Size of the test population, percent selected, selection differential, and the phenotypic variance of the test population for each cycle of selection for increased concentration† in the HOT and LOT population.

Cycle	Number progenies tested	Percent selected	Selection differential (S)‡	Phenotypic variance of tested population
		HOT¶ population		
C_a	88	13.6	0.37	0.70
C_0	52	26.9	0.60	0.35
C_1	na§	na	na	na
C_2	198	10.1	0.83	0.39
C_3	183	10.9	0.94	0.57
C_4	142	14.1	0.90	0.55
C_5	198	10.1	0.95	0.59
C_6	241	10.0	0.94	0.48
C_7	194	10.3	0.96	0.38
		LOT# population		
C_0	124	13.7	1.30	1.01
C_1	145	12.4	1.04	0.53
C_2	82	24.4	0.81	0.54
C_3	na	na	na	na

† Oil concentration was determined by nuclear magnetic resonance spectroscopy (NMR).
‡ S: [Mean of selected families (g 100g^{-1} seed)] – [mean of the test population (g 100g^{-1} seed)].
§ Data not available.
¶ HOT: Recurrent half-sib selection procedure for increased oil concentration in seeds using high-oil cultivar, Ransom, as a tester.
# LOT: Recurrent half-sib selection procedure for increased oil concentration in seeds using low-oil line, N80-2177-2, as a tester.

RESULTS AND DISCUSSION

Results suggest that recurrent selection using a high-oil tester for genotype evaluation was a successful procedure for increasing oil content. There was a significant linear increase ($p < 0.01$) in oil content from C_0 to C_7 in the HOT populations (Table 2). The average mean oil content across all three locations increased from 202 g kg^{-1} in C_0 to 211 in C_7 (Table 3), i.e., 4.5%. Although there was a significant quadratic selection response ($p < 0.05$) due to a plateau after cycle 5, the increase was primarily linear ($p < 0.001$) at an average rate of 1.1 ± 0.2 g kg^{-1} per cycle (Table 2). Priadi (1993) used recurrent half-sib family selection to increase oil in a population of similar genetic origin to the one in this study. He showed improvements of only 0.5 ± 0.2 g kg^{-1} per cycle, suggesting that recurrent selection using testcross evaluation is the more effective procedure of the two methods.

Partition of the significant genotype × location interaction showed the effect to be due to quadratic response × location interaction (Table 2). The quadratic trend was most noticeable at Plymouth, where mean oil content increased from 195 g kg^{-1} in C_0 to 208 in C_6 and then dropped to 202 in C_7. Spearman's rank analysis indicated that there was no significant difference in rank for C_0 through C_7 among the three locations. Therefore, this interaction was due to changes in magnitude rather than in rank. There were highly significant location effects ($p < 0.01$) on oil content (Table 2). The average oil content for Clayton, Plymouth, and Sandhills were 208, 202, and 214 g kg^{-1}, respectively.

One possible reason for the plateau at C_6 and C_7 in our experiment may have been small effective population size and low initial genetic variability. Dudley and

Table 2. Combined analysis of variance over locations and cycles of selection for oil and protein concentration (g kg^{-1} seed) in the HOT, LOT, and hybrid populations.

Source	df	Mean squares	
		Oil	Protein
Location (Loc.)	2	1 320**	8 785**
Rep/Loc.	6	93	320
Genotype	17	77**	251*
HOT population†	7	81**	188
Linear	1	408**	442
Quadratic	1	106*	411
Lack of fit (Lof)	5	4	88
HOT sib hybrid§	3	31	36
HOT tester hybrid§	(3)	24	46
LOT population‡	3	38	32
LOT sib hybrid	1	3	3
LOT tester hybrid§	(1)	0	88
HOT vs. LOT	3	182**	811**
Checks¶	2	18 711**	56 106**
Genotype × Loc.#	34	19*	116
HOT pop. × Loc.	14	21	145
Linear × Loc.	2	11	23
Quadratic × Loc.	2	38*	472**
Lof × Loc.	10	19	88
HOT hybrid × Loc.	6	26	144
HOT test hybrid × Loc.	(6)	20	54
LOT pop. × Loc.	6	15	63
LOT hybrid × Loc.	2	8	32
LOT test hybrid × Loc.	(2)	36*	117
Loc. × (HOT vs. LOT)	6	20	105
Checks × Loc.	4	85**	1 167**
Error††	113‡‡	12	82

* Significant at 0.05 level.
** Significant at 0.01 level
† HOT: Recurrent half-sib selection procedure for percent seed oil using high-oil cultivar, Ransom, as a tester.
‡ LOT: Recurrent half-sib selection procedure for percent seed oil using low-oil line, N80-2177-2, as a tester.
§ Oil and protein content for tester hybrids were analyzed separately using the corresponding sib-hybrid seeds.
¶ Checks (Ransom, N80-2177-2, and C_a population) were included in the initial combined ANOVA to permit comparison with the checks and then were deleted from the ANOVA to examine the genotype effects.
# Error term for testing the genotype effects.
†† Error term for testing the genotype × location effects.
‡‡ The error degrees of freedom for protein was 116.

Lambert (1992) estimated that the number of genes affecting oil content for maize (*Zea mays* L.) was 69. Rawlings (1980) found that for short-term experiments of 5 to 10 cycles, expected progress began to plateau when the number selected was 16. This is larger than our initial samples. In our selection process, 12 plants were selected from 88 plants tested in the base population and 14 plants were selected from 52 plants tested in the C_0 population. Although the number of genes controlling oil content in soybeans is not known, those small testing and sampling sizes in the early cycles of selection may have limited the genetic background for our base population and caused the plateau at C_6 and C_7 and the low overall progress. Compared with recurrent selection for oil content in maize (Smith, 1908; Dudley and Lambert, 1992), where oil content ranged from 38.4 to 60.2 g kg^{-1} for the 163 ears selected in the base selection population, the oil content in our base population ranged from 206 to 243 g kg^{-1}. Our population had seven parents, but one of those, the male-sterile source, contributed half of the genetic make-up. So the genetic background might not have been as broad as Burr's White, the base population for the selection of maize oil content. This could be another reason for the lower rate of progress in our program.

Unexpected responses to recurrent selection in soybean as well as in maize have also been reported previously for other traits. Kenworthy and Brim (1979) found a significant yield improvement when selecting for seed yield per se but no significant linear response when selecting for efficiency expressed as the ratio of seed weight to straw weight or for an equally weighted rank index of yield and efficiency. Brim and Burton (1979) found a significant yield decrease in one population and a significant yield increase in another when selecting for percentage of seed protein. Comparing 90 generations of recurrent half-sib family selection for oil and protein in maize (Smith, 1908; Winter, 1929; Dudley et al., 1974; Dudley and Lambert, 1992), changes in oil or protein content were not strictly linear. Both plateaus and decreases were observed while selecting for high oil and plateaus and increases were observed while selecting for low oil for one or several consecutive generations. Therefore, other factors must influence selection results. One of those factors may be environment. For example, Smith (1908) reported severe weather conditions, particularly rainfall, affected oil as well as protein content during the different selection years.

In contrast to the HOT program, oil content did not change significantly ($P > 0.05$) with selection in LOT populations (Table 2). The average oil content ranged from 202 g kg^{-1} in C_3 to 207 g kg^{-1} in C_2 but the differences were nonsignificant (Table 3). The significant increase in the HOT program and lack of increase in the LOT program indicates that choosing an appropriate tester is important for selection progress. Results suggest that the tester should represent the desired phenotype of selection, which is assumed to have a high frequency of favorable allelic genes. Similar results were obtained by Lewers et al. (1998). In their research, St. Martin and Aslam (1986) and Reese et al. (1988) found the choice of tester not to be critical for parental evaluation. However, the testers used in those studies were all high yielding lines. Therefore, one reason for this discrepancy may be that high yielding testers were not compared with use of low yielding testers in those experiments.

In our study, there were significant location effects for all other traits recorded except flowering date (data not shown). Genotype × location interaction was not significant for protein content, plant height, and seed yield, but was significant for flowering and maturity date, plant lodging, and seed weight (data not shown).

A highly significant negative simple correlation ($r = -0.88, p < 0.001$) was observed between oil and protein content. When combined over locations, mean protein content in HOT populations decreased from 400 to 391 g kg^{-1} from C_0 to C_7 as oil content increased from 202 to 211 g kg^{-1} (Table 3). The decrease was linear ($p < 0.05$) at a rate of -1.2 ± 0.6 g kg^{-1} per cycle. Protein content increased slightly, as oil content remained steady in C_6 and C_7. From C_0 to C_5, oil content increased linearly at a rate of 1.7 ± 0.2 g kg^{-1} per cycle and protein content

Table 3. Mean agronomic performance of each cycle of selection for the HOT and LOT populations.

Cycle	Oil†	Protein†	Flowering‡	Maturity§	Plant lodging	Plant height	Seed yield	Seed weight
	g kg^{-1} seed				Score#	cm	kg ha^{-1}	g (100 seed)$^{-1}$
HOT population††								
C_a	205	403	8	27	2.2	101	2498	15.4
C_0	202	400	9	27	2.2	101	2780	15.2
C_1	205	396	9	26	2.4	100	2454	14.8
C_2	208	395	10	27	2.5	96	2504	14.9
C_3	209	394	9	27	2.5	95	2618	15.2
C_4	209	389	9	27	2.4	97	2730	16.0
C_5	211	386	11	27	2.7	90	2733	15.5
C_6	210	395	10	27	2.3	97	2692	16.2
C_7	211	391	10	27	2.4	100	2831	15.2
LSD(0.05)	5	ns	ns	ns	ns	ns	ns	ns
LOT population‡‡								
C_0	206	404	10	27	2.5	100	2582	15.3
C_1	204	401	10	27	2.4	105	2687	15.1
C_2	207	400	10	27	2.5	99	2611	14.9
C_3	202	404	12	27	2.4	108	2581	15.3
LSD(0.05)	ns	ns	ns	ns	ns	ns	ns	ns
N80-2177-2	131	525	14	24	1.9	99	2208	16.2
Ransom	215	395	7	26	1.6	98	3437	16.8

ns, not significant
† Oil and protein was determined by near-infrared reflectance spectroscopy (NIR).
‡ The number of days from August 1 to flowering.
§ The number of days from October 1 to harvest maturity.
# Scored from 1 (no plants lodged) to 5 (all plants lodged).
†† HOT: Recurrent half-sib selection procedure for percent seed oil using the high-oil cultivar, Ransom, as a tester.
‡‡ LOT: Recurrent half-sib selection procedure for percent seed oil using the low-oil line, N80-2177-2, as a tester.

decreased linearly at a rate of -2.7 ± 0.3 per cycle. In this case, the ratio of change in oil to change in protein was $1.7/2.7 = 0.63$. Combined over all seven cycles and three locations, the ratio of change in oil to change in protein was $1.1/1.2 = 0.92$. The large negative correlation indicates that a dynamic equilibrium between the two may exist and further illustrates the well-established negative relationship between concentrations of seed protein and seed oil. The 0.63 ratio of change in oil to change in protein from C_0 to C_5 is similar to a previous estimate by Burton and Brim (1981). The negative correlation between oil and protein content was also indicated for hybrids in this experiment. If there was a positive midparent heterosis for oil content, there was usually negative midparent heterosis for protein content (Table 4).

The alteration of oil content in soybean seeds in this experiment produced no correlated changes in plant lodging, height, dates of flowering and maturity, seed weight, and yield over locations and cycles in either HOT or LOT populations. There were no significant changes in any of the traits measured in the LOT population (data not shown).

There was no significant ($p < 0.05$) change in oil content among cycles for tester hybrids or sib hybrids in either the HOT or the LOT populations (Table 2). For HOT populations, Ransom hybrid mean oil content ranged from 213 g kg^{-1} in cycle four to 216 in cycle six but decreased to 212 in cycle seven; sib hybrid-oil content increased from 204 to 210. For LOT populations, oil content in N80-2177-2 hybrid and in sib hybrids remained the same for the two cycles of selection at the levels of 175 and 203 g kg^{-1}, respectively.

Compared with selfed and sib hybrids in the HOT populations combined over cycles and locations, Ransom hybrids had a significantly ($p < 0.01$) higher paired difference in oil content of 0.4 and 0.5, respectively (Table 4). For LOT populations, the results were just the opposite. Tester N80-2177-2 hybrid had significantly ($p < 0.01$) lower paired difference in oil content of 3.0 and 2.9 compared to its corresponding selfed- and sib-hybrid populations (Table 4).

There was significant ($p < 0.01$) midparent heterosis combined over two cycles of selection for LOT popula-

Table 4. Mean paired differences (heterosis) of oil and protein content for tester hybrid and sib hybrid performance compared to midparent and selfed population performance in the HOT and LOT populations.

	Tester hybrid vs. midparent†‡		Tester hybrid vs. selfed population§		Sib hybrid vs. selfed population¶	
Cycle	Oil	Protein	Oil	Protein	Oil	Protein
HOT population#						
	g kg^{-1} seed					
C_4	1	1	4	4	−3	8**
C_5	1	1	3	6	−3	9
C_6	4	−7	6	−7	0	−3
C_7	−1	−4	2	−3	0	−1
Mean	1	−2	4**	0	−1	3
LOT population††						
	g kg^{-1} seed					
C_2	11*	−10	−32	59	−4	5
C_3	8*	−13*	−28	50	1	−2
Mean	9**	−12**	−30**	54**	−1	1

* Significant at 0.05 level.
** Significant at 0.01 level.
† Midparent = (Mean of selfed population + mean of corresponding tester)/2.
‡ Mean of paired difference of tester hybrid − midparent.
§ Mean of paired difference of tester hybrid − selfed population.
¶ Mean of paired difference of sib hybrid − selfed population.
# HOT: Recurrent half-sib selection procedure for percent seed oil using a high-oil cultivar, Ransom, as a tester.
†† LOT: Recurrent half-sib selection procedure for percent seed oil using a low-oil line, N80-2177-2, as a tester.

tions. Average midparent heterosis [tester-hybrid mean − (mean of selfed population + tester)/2], was 0.9 ($p < 0.001$) for oil content and –1.2 ($p < 0.01$) for protein content. For HOT populations, Ransom hybrids showed a nonsignificant average midparent heterosis of 0.1 in oil content, ranging from −0.1 to 0.4 (Table 4). Evidence of heterosis for soybean oil indicates that there may be some dominance effects for oil content. This finding is different from some previous research (Brim, 1973) but is consistent with results of Lewers et al. (1998). In the LOT populations, there were significant ($p < 0.05$) differences in sib-hybrid seed weight. There were no significant changes ($p < 0.05$) for any other sib-hybrid traits measured in either the HOT and LOT populations.

Recurrent half-sib selection has been widely used in maize as a way to improve the specific combining ability of a population (Hallauer and Miranda, 1981), but it has not been used in soybean. Because the soybean breeding product is a pure-line cultivar rather than an F_1 hybrid, selection for specific combining ability has never been a breeding goal. Yet, some soybean cultivars have been used successfully as a parent (e.g., Williams, Young, and Hutcheson) in the derivation of many productive cultivars. Thus it would seem that those cultivars have superior general combining ability. Using such a cultivar as tester in a recurrent half-sib selection program might be a way to improve the trait of interest, and improve the combining ability of the populations with the tester. Both favorable additive and additive × additive epistatic gene combinations would be accumulated. Once a desired change in the trait has been achieved, pure-lines with agronomic quality of the tester could be derived from the population × tester cross using modified pedigree selection.

Finally, this experiment further demonstrated that the use of nuclear male-sterility and a marker trait can be useful in facilitating a recurrent half-sib selection procedure that combines self-pollination and testcross production in the same season, as well as selfing and testcross evaluation in the same season. Each cycle of selection requires two seasons that could be completed in a single year if a winter nursery were used. Although environmental conditions may exert a significant effect on oil and protein contents, genotype × environment interactions were not significant for oil content in this study. Therefore, it is suggested that breeding progress for increased oil content can be made with progeny selection in a single environment.

APPENDIX

Ignoring genetic variation due to epistasis, the expected genetic progress per cycle after the recurrent half-sib selection procedure described in this paper is

$$\Delta G = S\ (3/8\ \sigma_A^2 + 9/32\ D_1)/\sigma_Y^2$$

where S is the selection differential, σ_A^2 is the additive genetic variance, D_1 is the covariance between additive and homozygous dominance effects, and σ_Y^2 is the phenotypic variance among the selection units. Both σ_A^2 and D_1 are defined in reference to the noninbred population in linkage equilibrium (Cockerham, 1983).

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